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Hormonal and behavioral patterns of reproduction in female hylobatids

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ABSTRACT

Ovarian cyclicity, reproductive behavior, and gestation length were characterized in female hylobatids using non-invasive fecal steroid analyses and behavioral data. Progestagen metabolites were quantified in fecal samples collected for 3 months from seven females housed at seven North American zoological institutions. Mean (±SEM) ovarian cycle length was 23.1 ± 1.5 days (seven females; 22 cycles) and was similar across individuals and species (range: 15.3–27.3 days). Gestation length in white-cheeked gibbons was 191 ± 7.0 days ($n = 2$), and female-initiated reproductive behavior occurred throughout the ovarian cycle for each individual. This was the first study of its kind to use fecal hormone metabolite analysis in combination with behavioral observations to characterize female reproductive traits for various hylobatid species. These results contribute to our general knowledge of the basic biology of hylobatids, highlight the importance of evaluating both hormonal and behavioral information, and assist the management and breeding of zoo-housed populations of these endangered primates to support overall conservation efforts.

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1. Introduction

Within the superfamily of apes known as the Hominoidea, the hylobatids comprise the largest family with four genera (*Nomascus*, *Hylobates*, *Symphalangus*, and *Hoolock*) and 13–15 described species (Fleagle, 1984; Groves, 2001). Gibbons and siamangs are believed to be primarily monogamous, forming long-lasting pair-bonds in the wild and zoos, a characteristic found in no other ape species, with the possible exception of humans, and only a few other primates (i.e., callitrichids, *Aotus* and *Callicebus* spp.). Hylobatid family groups usually include one to four offspring, with the breeding pair producing a single offspring approximately every 2–4 years (Leighton, 1987; Clutton-Brock, 1991; Geissmann, 1991; Palombit, 1992; Brockelman et al., 1998; MacDonald, 2001; Reichard and Barelli, 2008). Offspring of both sexes are thought to disperse from their natal group at sexual maturity, about 7–8 years of age (Dielentheis et al., 1991). Among all ape species, only siamangs (*Symphalangus syndactylus*) demonstrate direct paternal care in the form of infant-carrying beginning between 6 and 12 months post-partum (Chivers, 1974; Dielentheis et al., 1991; Dal Pra and Geissmann, 1994; Lappan, 2005).

Native to Southeast Asia and parts of India and China, hylobatids prefer various forest habitats (Chivers, 1972; Dao Van Tien, 1983; Palombit, 1992; Choudhury, 1996; Reichard and Sommer, 1997; Choudhury, 2006; Bartlett, 2009). All hylobatid species are considered either

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Table 1
Published data on ovarian cyclicity in hylobatids.

Species	Type of data	Number of females	Number of cycles	Estrous cycle length (± SEM)	Range (days)	Reference
Nomascus leucogenys	Menstruation	1	19	23.8 ± N/A	16–37	Bachmann and Sodaro (2001)
Nomascus leucogenys	Fecal hormones	1	38	21.9 ± 2.9	12–27	Lukas et al. (2002)[b]
Nomascus gabriellae	Urinary hormones	2	7	21.1 ± 1.2	19–22	Geissmann and Anzenberger (2009)
Symphalangus syndactylus	Menstruation/urinary hormones	1	12	21.8 ± 4.4	19–34	Knott et al. (1993)
Symphalangus syndactylus	Menstruation	N/A	N/A	28.0 ± N/A	N/A	Von Hegel, pers. comm. cited in Orgeldinger (1989) (p. 229)
Symphalangus syndactylus	Copulatory behavior	1	N/A	N/A	42–49	Alberts (1983)
Hylobates lar[a]	Menstruation, copulatory behavior, fecal hormones, genital swellings	84	577	24.7 ± 1.6	15–44	Carpenter (1941), Ellefson (1974), Breznock et al. (1977), Czekala et al. (1985), Chaiyabutr and Maharrnop (1987), Dahl and Nadler (1992), Nadler et al. (1993), Ebert (1999) and Barelli et al. (2007)

[a] Most papers have used this species, and numbers are mean values from several papers.
[b] Note that only Lukas et al. (2002) used fecal hormones in *Nomascus leucogenys*, and that most studies are based on only one female, except for *Hylobates lar*.

'endangered' or 'critically endangered' by the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species (IUCN, 2009), mainly as a consequence of habitat fragmentation and loss, intense hunting pressure, and human encroachment (Geissmann, 2000). Zoological institutions maintain populations of three species: white-cheeked gibbons (*Nomascus leucogenys*), siamangs (*S. syndactylus*), and white-handed gibbons (*Hylobates lar*), with other species represented by smaller numbers of individuals (Petersen et al., 2010a,b,c).

Given that hylobatids are the least studied yet only monogamous apes, a more complete understanding of basic reproductive physiology in these species can potentially contribute to our knowledge of the evolution of monogamy within apes and among primates more generally. The majority of previously published information on hylobatid reproduction has come from studies of the *Hylobates* genus, as demonstrated in Table 1. In contrast, only a few published studies have characterized reproduction in other hylobatid genera (i.e., *Nomascus* and *Symphalangus*), leaving a gap in the understanding of hylobatid reproductive physiology as a whole. Additionally, no studies to date have used fecal hormone metabolite analysis to characterize basic reproductive patterns within the *Symphalangus* genus (siamangs), and only one previous study (Lukas et al., 2002) has done so within the *Nomascus* genus (white-cheeked gibbons). Furthermore, no studies to date have used enzyme-immunoassay (EIA) to study reproduction in *Nomascus*. The use of feces is the most practical means of assessing endocrine patterns in both wild and zoo populations, primarily because of the ease and non-invasive nature of sample collection. Measuring steroid hormones in feces, rather than blood, is advantageous because it does not require animal immobilization and provides a pooled average of hormone values. It also allows for longitudinal assessment of reproductive activity over time (Brown et al., 1994). Furthermore, fecal hormone analysis and EIA are superior methods, primarily when it comes to field studies because they are more mobile, and therefore may be used to assess wild populations of endangered species (Santymire and Armstrong, 2010). Various measures of ovarian cycle length have been published for hylobatids (ranging from 21.1 to 28.0 days, depending on the species), but most of these studies were based on measures from a single individual (Table 1). Similarly, limited information is available regarding hylobatid gestation lengths, which have been reported for white-cheeked gibbons (211 days), white-handed gibbons (210 days), and siamangs (235 days). These values, however, were based on only a few individuals and, therefore, may not capture the full extent of variation in gestation length (Petersen et al., 2010a,b,c).

The aim of the present study was to characterize gonadal steroidogenic activity in zoo-housed gibbons and siamangs using non-invasive fecal hormone metabolite monitoring. Specific objectives were to: (1) characterize fundamental reproductive traits in female hylobatids of the genus *Nomascus* and *Symphalangus*; (2) determine variability in ovarian cyclicity within and among individuals and species and in gestation within one species, the white-cheeked gibbon (*N. leucogenys*); and (3) compare temporally matched reproductive behavior with hormonal metabolite patterns of ovarian cyclicity in female hylobatids.

2. Materials and methods

2.1. Animals

Seven adult female hylobatids (mean age: 18.1 ± 2.5, range: 11–30 years old, 5 *Nomascus* spp., 2 *Symphalangus*) were studied at institutions in North America, including Lincoln Park Zoo (Chicago, IL), Brookfield Zoo (Chicago, IL), Minnesota Zoological Garden (Apple Valley, MN), Busch Gardens (Tampa, FL), Woodland Park Zoo (Seattle, WA), Little Rock Zoo (Little Rock, AR), and Denver Zoo (Denver, CO). In addition to the two siamangs (SIA), the *Nomascus* spp.

Table 2
Reproductive history and housing information for adult female hylobatids included in the study and their male partners.

Hylobatid number	Species	Sex	Age (y)	Housing	On display	Proven breeding (parity)	Lighting
0200[a]	*N. leucogenys*	F	13	Paired	Yes	Yes (2)	Artificial (indoor)
0182	*N. leucogenys*	M	16				
0207	*N. leucogenys*	F	21	Paired	Yes	Yes (3)	Natural (outdoor) and artificial (indoor)
0223	*N. leucogenys*	M	20				
T008	*N. leucogenys*	F	11	Paired	Yes	No	Artificial (indoor)
T013	*N. leucogenys*	M	11				
0213[a]	*N. leucogenys*	F	21	Paired	Yes	Yes (3)	Natural (indoor) and artificial (indoor)
0176	*N. leucogenys*	M	25				
A01138[b]	*N. gabriellae*	F	13	Solitary after parturition	No	Yes (2)	Natural (outdoor) and artificial (indoor)
388[c]	*S. syndactylus*	F	18	Paired	Yes	No	Natural (outdoor) and artificial (indoor)
178	*S. syndactylus*	M	29.5				
485	*S. syndactylus*	M	7	Paired	Yes	No	Natural (outdoor) and artificial (indoor)
174	*S. syndactylus*	F	29.5	Paired	Yes	Yes (3)	Natural (outdoor) and artificial (indoor)
178	*S. syndactylus*	M	29.5				

[a] Females that became pregnant during study.
[b] Female was pregnant at the beginning of the study, but abandoned infant on day of parturition; singly housed afterward.
[c] Female was paired with one male (#178) at one institution, followed by a different male (#485), after transfer to another institution.

included four white-cheeked gibbons (*N. leucogenys*; WCG) and one buff-cheeked gibbon (*N. gabriellae*, BCG). All subjects were housed at institutions accredited by the Association of Zoos and Aquariums (AZA) and the project was conducted with approval from the University of Chicago IACUC (ACUP 71848). Animals were housed in pairs on or off public display with natural and/or artificial lighting (Table 2). Parity and breeding history varied across females.

2.2. Reproductive behavior

Behavioral data were collected for a subset of study females. All behavioral data were collected using a hylobatid ethogram modified from a pre-existing ethogram developed for the EthoTrak software program (Atsalis et al., 2005), and observers were trained by one of the authors (MR) until they reached greater than 90% inter-observer reliability (Crockett, 1996). The ethogram included behaviors related to reproduction and/or pair-bond maintenance, including the female-initiated behaviors of soliciting reproduction and presenting for reproduction. Data were collected on each pair using 1-min scan-sampling methods during 15-min observations and were collected two to five times per week over a 3-month period, or approximately 2–5 h per cycle (Altmann, 1974). All occurrences of reproductive behavior were analyzed to examine the temporal relationship between behavior and fecal hormonal metabolite patterns (Atsalis et al., 2004). Additionally, zoo keepers' notes on reproductive behavior were incorporated into the analyses, although instances of this were rare.

2.3. Ovarian cyclicity and gestation length

To assess basic hormonal characteristics of the ovarian cycle, all seven female hylobatids were monitored using fecal sample collection over a 3-month period. When possible daily samples were obtained to characterize basic reproductive traits of the ovarian cycle and validate gestation length. When daily collection was not feasible, samples were collected approximately four to five times per week.

Two of the seven females (both WCGs; #0200 and #0213) became pregnant over the course of the study. Fecal samples collected during gestation were used to determine the patterns of progestagen metabolites and gestation length. Fecal progestagen metabolite concentrations during pregnancy and the ovarian cycle were compared to assess hormonal differences across reproductive states.

2.4. Fecal sample processing

Fecal sample collection was concurrent with behavioral data collection and took place between June 2006 and November 2009. The length of data collection varied across females, ranging from 3 months to nearly 3 years; however, for each individual only 3 months of data were used for this study. Zoos used either green liquid food-coloring (Gordon Food Service, Tampa, FL) or blue gel food-coloring (Americolor, Placentia, CA) as fecal markers, administered in food items, and fresh fecal samples were uncontaminated by urine or substrate. Each fecal sample was placed into a sealed, plastic bag, and stored at −20 °C until shipped to the endocrinology laboratory at Lincoln Park Zoo's Davee Center for Epidemiology and Endocrinology for processing and analysis. Fecal samples were lyophilized (Thermo Modulyo Freeze Dryer, model D-115), then steroid metabolites were extracted from fecal samples using a method modified from Brown et al. (1994) to include agitation on a Glas-col mixer (Glas-col, Terre Haute, IN) on setting 60 for 30 min instead of boiling samples. Samples were then diluted to 1:20 for progestagens in dilution buffer (0.2 M NaH_2PO_4, 0.2 M Na_2HPO_4, NaCl) prior to analysis by enzyme

immunoassay for quantitative measurement of hormone metabolite concentrations.

2.5. Enzyme-immunoassay (EIA)

Fecal progestagen metabolites were measured using EIA. A progesterone EIA was validated for fecal extracts of each species here by demonstrating: (1) parallelism between binding inhibition curves of fecal extract dilutions (1:2–1:2048); and (2) significant recovery (>90%) of exogenous progesterone added to fecal extracts (WCG: 1:20; $y = 0.931x - 0.748$, $R^2 = 0.998$; BCG: 1:20; $y = 0.991x + 0.797$, $R^2 = 0.994$; SIA: 1:20; $y = 0.999x + 0.748$, $R^2 = 0.995$). From the parallelism data, the appropriate dilution at ~50% binding was prepared for progesterone (1:20). Progesterone monoclonal antiserum (CL425; provided by C. Munro, University of California, Davis, CA) was used at a dilution of 1:10,000. Horseradish peroxidase (HRP) was prepared for the pregnane EIA at a dilution of 1:40,000. Cross-reactivities of the progesterone antibody were: progesterone, 100%; 4-Pregnen-3α-ol-20-one, 188%; 4-Pregnen-3β-ol-20-one, 172%; 4-Pregnen-11α-ol-3,20-dione, 147%; 5α-Pregnan-3β-ol-20-one, 94%; 5α-Pregnan-3α-ol-20-one, 64%; 5α-Pregnan-3,20-dione, 55%; 5β-Pregnan-3β-ol-20-one, 12.5%; 5β-Pregnan-3,20-dione, 8%; 4-Pregnen-11β-ol-3,20-dione, 2.7%; 5β-Pregnan-3α-ol-20-one, 2.5%; pregnanediol, 5α-Pregnan-3α,20β-diol, androstenedione, and corticosterone, <0.1% (Graham et al., 2001). Assay sensitivity was 0.78 pg/well for progesterone, and intra- and inter-assay coefficients of variation were <10% and 15%, respectively.

2.6. Data analyses

An iterative process was used to calculate individual baselines for fecal progestagen metabolite concentrations for each female (Brown et al., 1994; Moreira et al., 2001). First, the mean progestagen metabolite value over the period of sample collection plus 1.5 standard deviations (SD) was calculated. All values that were greater than that value were removed and the mean plus 1.5 SD value was recalculated. This process continued until there were no more progestagen metabolite values exceeding the mean plus 1.5 SD value, and the resulting mean was designated as the individual baseline progestagen metabolite value. Any progestagen metabolite value exceeding the calculated baseline value plus 1.5 SD was considered an elevated value (Moreira et al., 2001).

The length of the luteal phase was defined as the number of consecutive days that fecal progestagen metabolite concentrations were elevated, excluding all single day increases or decreases in hormone metabolite concentrations. The greatest progestagen metabolite value during each luteal phase was considered the peak progestagen metabolite value. The end of a luteal phase was defined as a return of progestagen metabolites to baseline for at least 5 days. The length of the follicular phase was calculated by counting the number of days fecal progestagen metabolite concentrations were not elevated. The ovarian cycle length was defined as the number of days from the first elevated progestagen metabolite value until the first elevated progestagen metabolite value of the next cycle. When daily fecal sample collection was not feasible (i.e., missing data) and a hormone metabolite value dropped to baseline or jumped to an elevated level for only 1 day, it was considered a sampling error and ignored (Adkin et al., 2012). In most cases, sample collection began mid-cycle, preventing the simple addition of follicular and luteal phases to estimate overall length of the ovarian cycle. Therefore, mean ovarian cycle length was calculated separately from the mean of the two phases, so that the lengths of the follicular and luteal phases did not necessarily add up to overall cycle length.

Mean (±SEM) length of the luteal phase, follicular phase, and ovarian cycle was calculated for each individual, and overall means (±SEM) were also calculated for all individuals and each species. Given the small sample size of the present study, comparison of ovarian cycle length across individuals and across species using standard statistical tests would likely not have adequate power. Therefore, these data are presented using descriptive statistics.

Pregnancy was defined as the period of sustained elevated fecal progestagen metabolite concentrations (typically larger than peak progestagen metabolites during the ovarian cycle) prior to parturition, immediately followed by a drop in progestagen metabolite concentrations. Gestation length was calculated from the first of three consecutive days of an extended increase in fecal progestagen metabolite concentrations to the day of parturition based on information from each institution. Individual gestation length was estimated for each female, and an overall mean gestation length was calculated.

3. Results

3.1. Reproductive behavior

Over the course of 196 total observation sessions, females were observed 55 times either soliciting reproduction or presenting for reproduction, or 0.28 occurrences per observation session for all females. There was an almost equal distribution between the two behaviors for all females, and individual rates of reproductive behavior ranged from 0.06 to 0.84. Ovarian cycles were detectable in all seven adult female hylobatids, although some females' cycles were more distinct than others. An ovarian cycle profile for each female is shown in Fig. 1 (WCG: A–D represent #0200, #0207, #T008, #0213, respectively; BCG: E, #A01138; SIA: F and G represent #388 and #174, respectively). Every observed occurrence of reproductive behavior is shown on ovarian cycling profiles for all WCGs (Fig. 1A–D) and SIA #388 (Fig. 1F), but not for BCG #A01138 (Fig. 1E) or SIA #174 (Fig. 1G) because these females were not housed with a male during the data collection period. Among all females, regardless of species, reproductive behavior occurred throughout the ovarian cycle, during both luteal and follicular phases (Fig. 1A–D and F).

3.2. Gestation length

A fecal progestagen metabolite profile for one of the two WCG females (#0213) that became pregnant

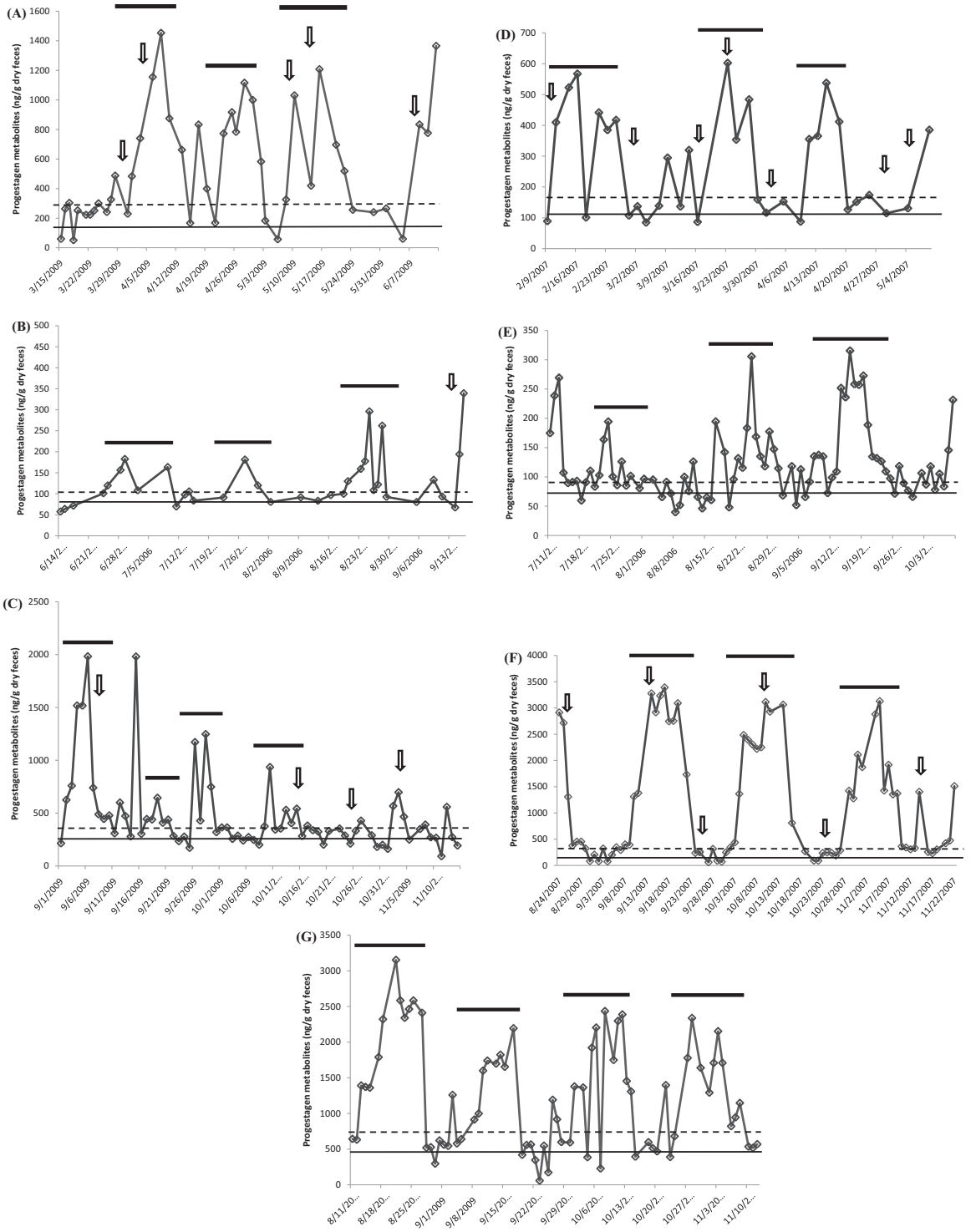

Fig. 1. Temporal pattern of reproductive behavior relative to ovarian cycle profiles for adult female hylobatids. Daily fecal progestagen (closed diamonds) metabolite concentrations for each female WCG (A–D represent #0200, #0207, #T008, #0213, respectively), BCG (E, #A01138), and SIA (F–G represent #388 and #174, respectively). Solid lines indicate baseline fecal progestagen metabolites, and dashed lines indicate threshold for elevated values. Note that the *y*-axis scales differ for WCGs and BCGs but are consistent for both SIAs. Occurrences of reproductive behavior (open arrows) are noted for those females that engaged in reproductive behavior (A–D, F), and solid black bars indicate duration of the luteal phase.

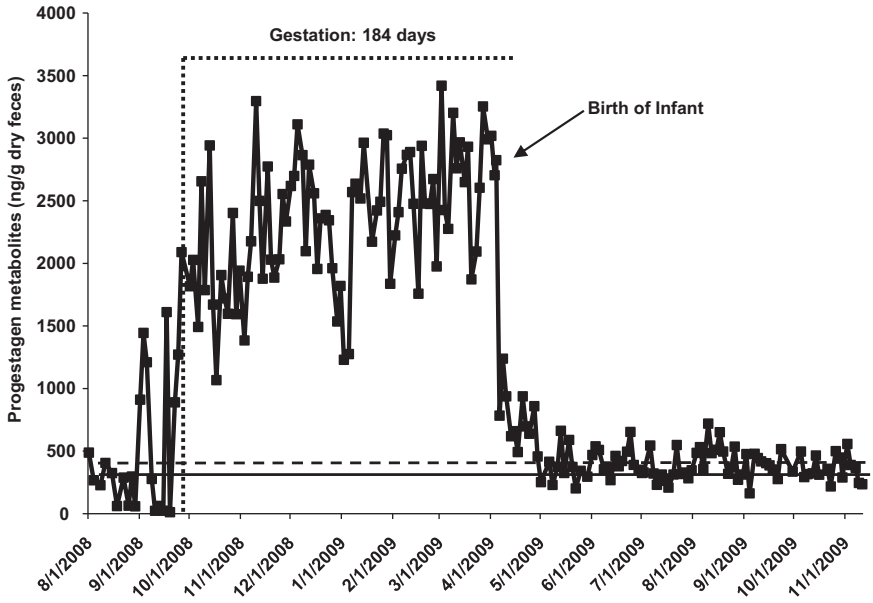

Fig. 2. Pregnancy profile in adult female WCG. Fecal progestagen metabolite concentrations for WCG #0213. Solid line indicates baseline of periods when ovarian cycles occurred and dashed line indicates elevated values (prior to and following pregnancy).

during the study is shown in Fig. 2. The profile depicts the entire duration of gestation and birth of the infant. Mean progestagen metabolite concentration during gestation was roughly seven-fold greater (2,334.4 ± 60.3 ng/g dry feces) than during ovarian cycling (309.1 ± 9.2 ng/g dry feces), and gestation length was estimated at around 184 ± 14 days (an error of 14 days is included for #0213 because of the difficulty associated with identifying the exact date of conception). Note the increase and subsequent sustained elevation of fecal progestagen metabolites and the steep decrease in progestagen metabolite concentrations immediately following parturition. Gestation length for the other WCG female (#0200), calculated from the estimated date of conception to the date of parturition (12/27/2009), was 198 days. The overall mean gestation length for the two female WCGs was 191.0 ± 7.0 days.

3.3. Ovarian cyclicity

Table 3 shows reproductive traits of the ovarian cycle in all seven adult female hylobatids. Overall mean peak progestagen metabolite concentration (1,294.7 ± 425.2 ng/g feces; range: 220.0–3111.5 ng/g feces) was approximately six-fold greater than baseline (207.7 ± 53.9 ng/g feces, mean range: 73.0–487.9 ng/g feces) (Table 3). Overall mean ovarian cycle length for all females was 23.1 ± 1.5 days (n = 7 females), and the mean ovarian cycle length across all cycles was 23.0 ± 1.1 days (n = 22 cycles; Table 3). Mean ovarian cycle length was 23.1 ± 2.5 days (n = 4; range: 15.8–27.3 days) for WCG, 24.0 days (n = 1) for BCG, and 23.0 ± 0.2 days (n = 2; range: 22.7–23.3 days) for SIA (Table 3).

4. Discussion

This study was the first to use fecal hormone metabolite analysis to characterize the ovarian cycle in SIAs (*Symphalangus* genus) and BCGs (*Nomascus* genus), and only the second to do so using any method in WCGs. Additionally, while EIA as a method of analysis has been employed in the genus *Hylobates*, this study was the first to characterize reproductive traits of WCGs (*Nomascus* genus) using EIA methodology. These methodologies may be more practical, especially with regard to field studies of endangered species, given their mobility. This was also the first study to document ovarian cycle and gestation lengths and associate hormonal patterns with reproductive behavior in female hylobatids more generally. Female-initiated reproductive behavior was also determined to occur throughout the ovarian cycle.

Ovarian cyclicity was detected in all seven female hylobatids, although some females' cycles were more distinct than others. For example, female SIA #388 had three very distinct ovarian cycles with distinct peaks in progestagen metabolites, whereas the other female SIA's (#174) profile was not as clearly defined. Similarly, although peaks in progestagen metabolite concentrations and ovarian cycling were still measurable in female WCGs and BCG, ovarian cycles were also less distinct. Lukas et al. (2002) presented data using radio-immunoassay (RIA) on a female WCG that demonstrated similarly indistinct patterns in the ovarian cycle. This result might be explained by variation in individual physiology or environmental factors, but continued analysis of the ovarian cycle across several individuals of different hylobatid species is needed to further elucidate explanations for variation in endocrine patterns.

Determination of seasonality in the seven female hylobatids was not possible in the present study. However, it

Table 3
Female hylobatid reproductive traits as assessed by longitudinal fecal hormone metabolite analysis; Values are means ± SEM; values in parentheses represent numbers of follicular or luteal phases or ovarian cycles (range also indicated).

Female	Duration of follicular phase (d)	Ovarian cycle length (d)	Duration of luteal phase (d)	Baseline progestagen (ng/g feces)	Mean progestagen/luteal phase (ng/g feces)	Peak progestagen/luteal phase (ng/g feces)
WCG #1 (0200)	10.0 ± 2.1 (range: 7–14; *n* = 3)	23.3 ± 2.4 (range: 17–27; *n* = 3)	13.3 ± 1.1 (range: 11–15; *n* = 3)	197.2 ± 23.8	847.9 ± 69.1	1259.1 ± 100.7
WCG #2 (0207)	16.0 ± 1.5 (range: 13–18; *n* = 3)	27.3 ± 0.7 (range: 26–28; *n* = 3)	11.3 ± 1.9 (range: 9–15; *n* = 3)	86.2 ± 3.1	163.3 ± 15.0	220.0 ± 37.9
WCG #3 (T008)	7.8 ± 2.2 (range: 6–14; *n* = 4)	15.8 ± 2.4 (range: 13–23; *n* = 4)	8.0 ± 0.6 (range: 7–9; *n* = 4)	255.5 ± 9.4	624.8 ± 66.1	1101.6 ± 244.8
WCG #4 (0213)	13.7 ± 1.9 (range: 10–16; *n* = 3)	26.0 ± 2.1 (range: 25–31; *n* = 3)	12.1 ± 1.5 (range: 10–15; *n* = 3)	120.9 ± 6.7	394.9 ± 36.0	569.1 ± 18.7
BCG #1 (A01138)	9.5 ± 1.4 (range: 7–12; *n* = 4)	24.0 ± 1.7 (range: 21–27; *n* = 3)	15.3 ± 0.9 (range: 14–17; *n* = 3)	73.0 ± 2.4	145.3 ± 10.3	271.8 ± 38.8
SIA #1 (388)	9.0 ± 0 (*n* = 4)	22.7 ± 1.5 (range: 21–24; *n* = 3)	13.7 ± 1.5 (range: 12–15; *n* = 3)	233.4 ± 16.9	2083.0 ± 157.7	3111.5 ± 149.3
SIA #2 (174)	10.3 ± 0.9 (range: 9–12; *n* = 3)	23.3 ± 0.7 (range: 22–24; *n* = 3)	13.0 ± 1.1 (range: 10–15; *n* = 4)	487.9 ± 26.0	1733.0 ± 97.7	2530.1 ± 213.4
Mean ± SEM	10.9 ± 1.1 (*n* = 7)	23.1 ± 1.5 (*n* = 7)	12.3 ± 1.0 (*n* = 7)	207.7 ± 53.9 (*n* = 7)	856.0 ± 289.7 (*n* = 7)	1294.7 ± 425.2 (*n* = 7)

should be noted that seasonal variation in breeding has not been reported in zoos or in the wild for this primate taxon (Barelli et al., 2008; Savini et al., 2008). Additionally, although fecal estrogen metabolites were measured in females using an estradiol-17β EIA, results were uninformative.

When reproductive behavior was temporally matched with progestagen metabolite concentrations for all four WCGs and one SIA during ovarian cycles, results demonstrated that reproductive behavior occurred throughout the ovarian cycle. Although females were only observed between 2 and 5 h per ovarian cycle, the results suggest that reproductive behavior may not be tightly linked to hormonal cyclicity in hylobatids, which, conversely has been documented in other apes, including lowland gorillas (Nadler and Collins, 1991; Patterson et al., 1991) and orangutans (Nadler, 1982). The fact that reproductive behavior was not confined to the fertile phase of the ovarian cycle suggests that such behavior may not be strictly functional for pregnancy and reproduction in the hylobatids. Mating throughout the ovarian cycle has also been documented in some New World monkey species, including many callitrichids (Kendrick and Dixson, 1983). Like the callitrichids, hylobatids are generally monogamous and form long-lasting pair-bonds. It is likely, therefore, that another function of reproductive behavior, similar to mutual allo-grooming or duetting, is to maintain the pair-bond within hylobatid pairs (Palombit, 1992; Geissmann and Braendle, 1997; Geissmann, 2000). The only other published data documenting female behavior throughout the ovarian cycle in zoo-housed gibbons (Lukas et al., 2002) excluded reproductive behavior from the study because it was thought to be too rare to measure. Lukas et al. (2002) only measured female social behavior (solicitation of grooming from the male), as well as contact and proximity to the male. These authors determined that the female WCG solicited grooming more from the male during the follicular phase than during the luteal phase, suggesting that this was a behavior confined to the period around ovulation. However, reproductive behavior and social behavior, such as grooming, are not interchangeable, especially because social behaviors like grooming are often beneficial for many other reasons related to health. Grooming, therefore, might be expected to occur throughout the female's ovarian cycle (Spruijt et al., 1992). It is also important to note that the findings presented in Lukas et al. (2002) were based on only one female gibbon, whereas this study included five females. Another potential explanation for seeing reproductive behavior throughout the ovarian cycle comes from studies of wild white-handed gibbons (*Hylobates lar*; WHG). Barelli et al. (2007) determined that within one wild population, females copulated during both fertile and not-fertile phases of the ovarian cycle, a pattern similar to the zoo-housed hylobatid females in the current study. Interestingly, both monogamy and polygamy existed within the wild population, and Barelli et al. (2007, 2008) suggested that reproductive behavior served as a way to confuse paternity if a female had multiple male sexual partners. Given that the hylobatids in the present study were housed together as monogamous pairs in zoos, it is impossible to evaluate whether this is the most appropriate explanation. It remains a possibility however, that

polygamy in hylobatids is actually more common in the wild than previously thought, and that paternity confusion remains a possible function of reproductive behavior. Whether the results presented here support strengthening of the pair-bond or paternity confusion, it might be expected that reproductive behavior would occur both during and outside of the fertile period in hylobatids.

Fecal progestagen metabolite profiles were compared during ovarian cycles and pregnancy in two WCGs to demonstrate differences in patterns between reproductive states. The most obvious and anticipated difference between these hormonal patterns is the abrupt increase and sustained elevation of progestagen metabolites during gestation, followed by a steep decrease in progestagen metabolites immediately following parturition, characteristic of pregnancy in many mammals, including primates (Carter, 1993).

In most female primates, including humans, gestation length is typically about nine times the length of the ovarian cycle (Rosenblatt, 1993). This appeared to hold true for the WCGs in the present study, with an overall mean gestation length of 191 days, nearly nine times the mean ovarian cycle length of 22.9 days. WCG #0213 had an estimated gestation length of 184 ± 14 days. In this female, progestagen metabolite concentrations increased then fluctuated prior to remaining elevated for a sustained period of time. The exact date of conception, and, therefore, the actual gestation length, for this female was difficult to pinpoint. A gestation length of 184 days is somewhat shorter than previous reports of 211 days (Petersen et al., 2010a), and adding a 2 week margin of error to this female's pregnancy, which is common practice even for human mothers, would lengthen it to 198 days. Estimated gestation length for WCG #0200 was 198 days, which is closer to published reports. The increase in progestagen metabolite concentrations at conception was much clearer for this female, and so the data are likely more reliable. It has been reported that gestation lengths for the WHG and the SIA are 210 days and 235 days, respectively (Petersen et al., 2010a,b). To date, there has been limited information on gestation length in hylobatids. Differences among the findings here and previous reports of gestation length among hylobatids may relate to the anecdotal nature of earlier estimates, especially because most of these reported values were based on visual cues and not hormone metabolite monitoring.

The overall mean ovarian cycle length across female hylobatids in the present study was 23.1 ± 1.5 days (Table 1), which is similar to previous reports (Orgeldinger, 1989; Knott et al., 1993; Geissmann and Anzenberger, 2009). Interestingly, the mean ovarian cycle length across all cycles (23.0 ± 1.1 days) was very close to the mean across females, and there was even less variability across ovarian cycles. Even with small sample sizes, species-specific findings from this study are in agreement with previous reports, indicating a fairly small amount of variation in ovarian cycle length among individuals. Mean ovarian cycle length for WCGs in this study (22.9 days) was similar to findings from the only two published values to date, 23.8 days (Bachmann and Sodaro, 2001) and 21.9 days (Lukas et al., 2002). A mean ovarian cycle length of 24.0 days for the BCG was similar to the only other reported value of 21.1 days (Geissmann and Anzenberger, 2009).

Variation in ovarian cycle length within an individual was minimal, except for WCG #0213. Among individuals, it is important to point out that WCG #T008 had a much shorter overall ovarian cycle length than other females in the present study. Although this result was not significant and may have been due to the small sample size, it is nevertheless somewhat anomalous. Her very short ovarian cycle length may be related to her age, parity, or a recent pairing with a new male. In addition to being the youngest female in the study, she was also nulliparous and had recently been moved to another institution with her mate. Shortly after she was moved, however, her mate died, and she was then paired with a new male. Data collected for this study began only 7 months after this pairing. Interestingly, Nuss (2009) determined that female Goeldi's monkeys (*Callimico goeldii*) suppressed ovarian cycling when paired with a new, unfamiliar male. One or all these factors could have had an effect on this female's ovarian cycle.

Although it was not possible to statistically test for differences in ovarian cycle length across individuals or across species due to the small sample size of the present study, cycle lengths appear to be similar across individuals, and one might assume that great variation is unlikely.

5. Conclusions

This study provides the first characterization of ovarian cycle traits for three species of hylobatids (SIA, BCG, and WCG) using fecal hormone metabolite analysis and EIA. These results provide the first preliminary evidence to address questions of the evolution of reproductive traits across hylobatids, given this taxon's unique phylogenetic position. Though the small sample size for this study necessitates cautious interpretation of results, the findings presented here do suggest that reproductive behavior occurs throughout the ovarian cycle, and therefore may serve as a mechanism to maintain strong pair-bonds. As one might expect, the length of the ovarian cycle appeared to be similar across all individuals and across species, and supports the relatively recent divergence of these taxa from one another. In addition, ecological similarities suggest that there should not be strong selective pressures leading to systematic differences in ovarian cycle or gestation lengths among the taxa. The findings presented here also have potential practical value. The use of a reliable noninvasive approach to hormonal monitoring is advantageous in endangered species like the hylobatids, whose wild populations have been drastically reduced. More accurate hormonal monitoring can provide opportunities for more precise timing of breeding and increased reproductive success in zoo-housed hylobatid populations. Additionally, a clearer picture of hylobatid reproductive physiology will allow for more precise monitoring of pregnancy. Taken together, the results from this study contribute to the understanding of the evolution of reproductive patterns in the Hylobatidae and enhance the knowledge base for further investigation of hylobatid reproduction and conservation.

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